THE CANADIAN VETERINARY JOURNAL
LA REVUE VÉTÉRINAIRE CANADIENNE
Volume 13 December-décembre 1972 No. 12

EFFICACY OF AN INTRAMUSCULAR INFECTIOUS BOVINE RHINOTRACHEITIS VACCINE AGAINST ABORTION DUE TO THE VIRUS

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Introduction

It has been known for several years that the virus which causes infectious bovine rhinotracheitis (IBR) in cattle can also cause abortion in non-immune cows or heifers (3, 5, 7, 9, 13). Such abortions have become widespread in beef cattle in Western Canada only within the past two or three years (15).

In North America, attenuated IBR virus vaccines for intramuscular injection have been widely advocated and widely used to protect against the respiratory form of the disease (1, 11). However, the authors could not find documented evidence to support the hypothesis that the administration of such vaccines to cattle before breeding would protect them against abortion as a result of exposure to 'field' strains of IBR virus. Therefore, in 1970–71 the experiment described here was initiated in an attempt to provide better answers to the questions often raised by producers and veterinarians alike in regards to the uses and limitations of these vaccines in preventing abortion.

Materials and Methods

Experimental Procedures

Forty non-pregnant heifers, two years old and seronegative for IBR antibody, were purchased on September 17, 1970 (day 1). They were maintained in conventional feedlot pens on a balanced ration for the duration of this experiment.

On day 16 these heifers were randomly allotted to three pens (separated) as follows:

Pen A – Controls (not vaccinated) – 20 heifers

Pen B – Vaccinated once on day 16 – 10 heifers

Pen C – Vaccinated twice on days 16 and 32 – 10 heifers

These animals were maintained in the above pens until 16 days after vaccination. The control and vaccinated heifers were then run together and bred by natural service. Seven of the animals, that remained non-pregnant, were removed from the experiment on day 167.

Then the remaining 33 heifers were segregated into three groups (I, II, III; see Table I) based on the stage of pregnacy. They were subsequently challenged intramuscularly or intranasally with a virulent field strain of IBR virus (Table I). The heifers in Groups I and II were challenged on day 175 and those in Group III on day 235.

TABLE I

EXPERIMENTAL DESIGN

	GROUP I			GROUP II			GROUP III		
Stage of Pregnancy When Challenged	3 months			4½ months			6 months		
Vaccination Program	Controls	Vaccinated Once	Vaccinated Twice	Controls	Vaccinated Once	Vaccinated Twice	Controls	Vaccinated Once	Vaccinated Twice
Number of animals	5	3	3	5	3	3	6	2	3
Animals Challenged Intranasally	3	2	2	3	2	2	3	1	2
Animals Challenged Intramuscularly	2	1	1	2	1	1	3	1	1

After challenge the animals were examined clinically twice daily for two weeks and body temperatures and any abnormal clinical findings were recorded. Following this two week period, observations were continued twice daily for an additional six weeks for signs of abortion. The heifers were kept in their respective groups for approximately one month

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following abortion or normal parturition. The study ended on day 372 (September 25, 1971).

Viruses

The IBR virus vaccine[1] was injected intramuscularly according to the manufacturer's directions.

Either of two different strains (P8 strain – isolated from a respiratory infection in dairy cows; V11 strain – isolated from an aborted fetus) served to challenge the immunity of the vaccinated and control heifers. The P8 strain, at the 6th passage in fetal calf kidney (FCK) cell cultures contained $10^{6.7}$ tissue culture doses 50% (TCD_{50})/ml. The V11 strain, at the 2nd passage in FCK cell cultures contained $10^{5.54}$ TCD_{50}/ml. The challenge inoculum, consisting of 2 ml of fresh viral culture mixed with 3 ml of Hank's balanced salts solution was administered either intranasally, with a 10 cm plastic cannula fitted over a needle (2.5 cm 16 gauge) on a syringe, or intramuscularly.

Collection of Specimens

For the assay of virus-neutralizing antibody, blood samples and nasal washings (done with 20 ml of 0.85% NaCl solution) were collected from the heifers prior to and after: (1) vaccination; (2) challenge; and (3) abortion or parturition. For virus isolation studies nasal swabs were obtained from all heifers just before vaccination or before challenge. From heifers in Group III, following challenge, nasal swabs were routinely taken every second day for two weeks.

Fetuses were obtained (when possible) from all heifers that aborted following challenge. All available fetuses were examined for gross and histopathological lesions and virological studies were done on appropriate tissues. Calves that died at parturition were similarly examined. Nasal swabs and precolostral and post-colostral blood samples were collected from some live, neonatal calves.

Isolation of Virus

Secondary monolayer tube cultures of FCK cells (2nd to 4th passage) were used for isolation of IBR virus from swabs or suspensions of tissue (6). The specimens were passed twice in FCK cultures.

Virus Neutralization (VN) Tests

All tests were done in FCK cell cultures by a micro-plate[2] method (12), serial two-fold dilutions of serum or undiluted nasal washings being reacted with 100 TCD_{50} of the P8 strain of IBR virus (14). Final readings were recorded after four days incubation.

Results

Clinical Observations

No clinical abnormalities were observed in the heifers following vaccination.

The intranasal inoculation of IBR virus resulted in the classical respiratory signs of IBR in the controls and, to a lesser degree, in the IBR-vaccinated heifers. The animals became anorectic and depressed 48 hours after inoculation. Respiratory rates increased and tracheal sounds became louder without evidence of pneumonia. The febrile response following the viral challenge was recorded (Figure 1). The clinical appearance of most animals returned to normal by the 5th to 7th day postinoculation. However, the fever, anorexia and depression persisted in two control heifers; by the 8th day postinoculation a severe dyspnea, associated with a necrotizing tracheitis, necessitated parenteral treatment with antibiotics for three days. The two animals responded quickly to this treatment and appeared to be clinically normal five days later.

Intramuscular challenge of immunity with IBR virus, as compared to intranasal challenge, gave a shorter and milder clinical disease in both control and vaccinated animals.

Abortion

A significant difference in abortion rates existed between the control heifers (10 of 16 or 62.5%) and the vaccinated heifers (1 of 17 or 5.9%) (Table II). The incidence of abortion was not significantly affected by factors such as the route of challenge inoculation, the strain of virus or the stage of pregnancy. Abortions occurred in: (a) five of seven control heifers after intramuscular challenge; and (b) in five of nine controls as well as one of 11 vaccinated heifers after intranasal challenge. The P8 strain of IBR virus caused abortions in three of six control animals. The V11 strain of virus caused abortions in seven of ten control heifers as well as one vaccinated heifer.

The 22 heifers, which had not aborted following challenge inoculation, produced nor-

[1]R4-1268, Modified Live Virus, Porcine Tissue Culture Origin, Connaught Medical Research Laboratories, Willowdale, Ontario, Canada.

[2]Falcon Plastics; a division of Becton, Dickinson and Co. (Canada) Ltd., Mississauga, Ont., Canada.

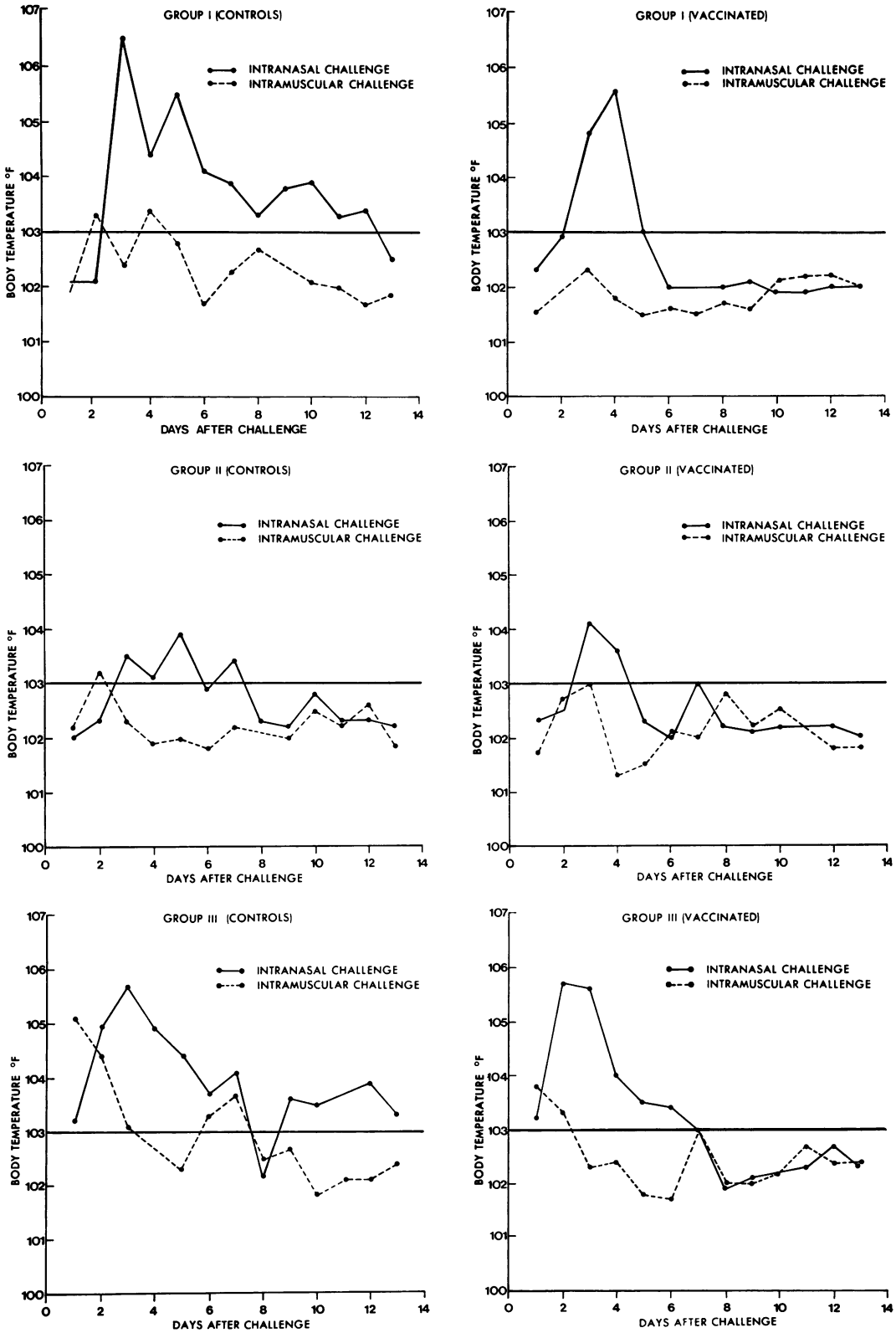

FIGURE 1. Comparison of daily mean temperatures of control and vaccinated heifers following intranasal or intramuscular inoculation of IBR virus.

TABLE II

FREQUENCY AND TIME OF ABORTION IN IBR—VACCINATED AND CONTROL HEIFERS FOLLOWING INTRANASAL OR INTRAMUSCULAR CHALLENGE

Group Number		Number of Abortions	Abortion at Days Post Challenge
I	Vaccinated	0/6*	—
	Controls.	3/5	14; 24; 21—30**
II	Vaccinated	0/6	—
	Controls	3/5	15; 22; 21—35**
III	Vaccinated	1/5	16
	Controls	4/6	8; 10; 17; 41
TOTALS:	VACCINATED	1/17 (5.9%)	
	CONTROLS	10/16 (62.5%)	

* Number aborting/numbers heifers inoculated.

**Range in days (fetus not found).

14 Numbers underlined indicate animals challenged intramuscularly.

TABLE III

ISOLATIONS OF IBR VIRUS FROM ABORTED FOETUSES AND TERM CALVES DELIVERED FROM HEIFERS (VACCINATES AND CONTROLS) FOLLOWING IBR CHALLENGE

Specimens	Isolations of Virus
Tissues (11 fetuses)	
1 (vaccinated group)	0/1*
10 (control group)	3/8
**Nasal swabs or tissues (22 term calves)	
16 (vaccinated group)	0/8
6 (control group)	0/4

*Number positive/number tested.

**Nasal swabs from 7 live calves on day of birth; tissues from 5 calves dead as result of prolonged, difficult parturition.

mal calves at term. Five of these calves died during difficult parturitions.

Virological Findings

The IBR virus was not isolated from nasal swabs from any of the heifers before vaccination, before breeding or before challenge of immunity. During the two weeks following intranasal or intramuscular challenge the virus was isolated from nasal swabs from vaccinated as well as control animals of Group III. In this group, the intranasally-challenged, control animals shed virus for a longer period of time (10 to 14 days) than either the intramuscularly-challenged, control animals or the vaccinated animals (challenged intranasally or intramuscularly).

The IBR virus was isolated from only three of the nine aborted fetuses (or portions thereof) available for study (Table III). The virus was isolated from the placenta in all three cases but from fetal tissues in only one case.

Seven fetuses were intact and suitable for necropsy, although only five of these were examined histologically. Pertinent gross observations were: (a) advanced autolysis in six fetuses; (b) increased peritoneal and pleural fluids in four fetuses; and (c) perirenal haemorrhages in four fetuses. A specific histological lesion seen in livers of three of the five fetuses examined was focal necrosis in which hepatocytes containing eosinophilic intranuclear inclusion bodies were scattered.

The five dead calves (of the 22 delivered at term, see Table III) had no significant lesions attributable to an *in utero* infection with IBR virus and the virus was not isolated from their tissues. In addition, nasal swabs taken from seven live calves on the day of birth were negative for IBR virus.

Serological Findings

The mean VN antibody titres of the 16 control and 17 vaccinated heifers are shown in Figure 2. All the control animals remained seronegative prior to challenge of their immunity. Three of the 17 vaccinated heifers failed to develop detectable serum antibody (at 1:2 dilution) following vaccination; one of the three animals developed a severe respiratory infection following intranasal exposure to IBR virus, subsequently aborted and developed a convalescent VN titre of 1:256. None of the 17 vaccinated animals developed a VN titre of greater than 1:8 after vaccination. These titres had generally declined to 1:2 or less by the time of challenge (5 to 7 months after vaccination).

After challenge of immunity all 33 pregnant animals developed IBR serum antibody titres ranging from 1:8 to 1:512. The vaccinated animals, as a group, developed higher VN titres than did the control animals. As can be seen from Figure 2 the intranasally challenged animals developed higher VN titres than did the intramuscularly challenged ones.

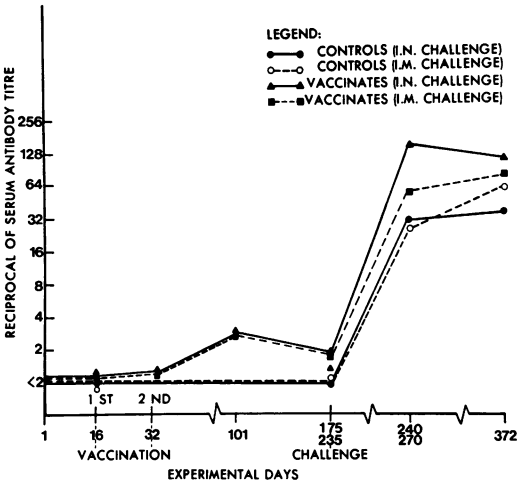

FIGURE 2. Mean titres of serum antibody to IBR virus in vaccinated and control heifers following vaccination and challenge.

TABLE iV

IBR SERUM ANTIBODY TITRES OF NEONATAL CALVES PRIOR TO AND AFTER INGESTION OF COLOSTRUM FROM IMMUNE DAMS

Calf Number	Serum Titres		
	Pre-colostral	Post-colostral	Dam
1.	<2	32	64*
2.	<2	8	128
3.	<2	8	128
4.	<2	32	64
5.	<2	128	128

*Reciprocal of serum dilution

Antibody to IBR virus was not detected in nasal washings of control or vaccinated animals prior to challenge of their immunity. At one to two months following challenge, nasal washings from intranasally-challenged control (2 of 11 tested) and vaccinated (5 of 17 tested) animals contained detectable antibody.

Table IV shows the serum antibody titres against IBR virus of five heifers and their newborn calves (before and after ingestion of colostrum). None of the calves had detectable antibody before nursing but all five acquired antibody as a result of nursing.

DISCUSSION

The vaccine provided adequate protection against abortion following experimental infection. Since only one of 17 vaccinated heifers aborted following experimental challenge of immunity it is quite possible that less severe, natural challenge may not cause abortion. Certainly a 5% abortion rate with vaccination is much preferred by a producer to a 30 to 60% abortion rate without vaccination.

Vaccination also reduced the severity and duration of respiratory disease following challenge, thus agreeing with results of other studies (2, 6, 16).

No information was obtained from this study on the 'escape' of vaccine virus from vaccinated animals during the two weeks after vaccination. However, as judged by negative VN tests there was no spread of vaccine virus from vaccinated to control heifers following mixing of the animals on the 16th day after vaccination.

Both challenge strains of virus, whether isolated originally from the respiratory tract or from an aborted fetus, were able to cause abortion. The stage of gestation (3, 4½ or 6 months) had no apparent association with susceptibility to abortion.

Two of the 11 abortions (10 controls, 1 vaccinated) occurred on days 8 and 10 following intranasal challenge. This is a few days earlier than reported by other investigators (4, 9). In both cases, the aborting heifers were still febrile and had a respiratory infection; both fetuses had gross and microscopic lesions typical of IBR infection and virus was isolated from one of them. The other nine abortions occurred following recovery from the clinical disease.

The gross and histopathological lesions observed in aborted fetuses are consistent with those previously reported (4, 8). The greater success in isolating IBR virus from the placenta as compared to fetal tissues has also been previously stressed (4, 8, 10). The inability to detect congenital defects or serum antibody to IBR virus in term calves supports the general theory that IBR virus has an 'all or none' effect on the embryo or fetus (4).

The IBR vaccine used here resulted in seroconversion of most vaccinated animals, although the titres of antibody are somewhat lower than in two other studies (6, 16). In spite of a decline in VN titres between vaccination and challenge of immunity the vaccinated animals apparently responded with an anamnestic or secondary response that was effective in preventing spread of virus across the placenta to the fetus. Whether this pro-

tective mechanism was due to nasal antibody, serum antibody or other factors is not completely understood.

The intranasally administered IBR vaccines (16) have some claimed advantages over the intramuscular vaccines in safety and more rapid onset of protection. However, since the intramuscular vaccines will prevent abortion and minimize respiratory disease, they should not be discarded.

Summary

The immunity of 17 IBR-vaccinated (intramuscular vaccine) and 16 control, pregnant heifers was challenged by the intranasal or intramuscular inoculation of IBR virus. Ten (62.5%) of the control animals aborted as compared to only one (5.9%) of the vaccinates.

Résumé

L'immunité de 17 taures gestantes vacciniés par la voie intramusculaire contre la rhinotrachéite infectieuse bovine (IBR) et de 16 taures gestantes témoins a été eprouvée par l'inoculation intranasale ou intramusculaire de virus IBR. Le taux d'avortement atteignit 62.5% (10 taures sur 16) chez les animaux non vaccinés, mais seulement 5.9% (une taure sur 17) chez les animaux vaccinés.

Acknowledgments

Financial support of the Alberta Cattle Commission, the Alberta Agricultural Research Trust and the Saskatchewan Horned Cattle Trust Fund is gratefully acknowledged.

The authors wish to thank: a) Dr. H. H. Nicholson of the Department of Animal Science for his cooperation in maintaining the animals; b) members of the faculty of the Department of Veterinary Pathology for performing the necropsies; c) Dr. David Hines and Mr. Mervin Wetzstein for observing the animals and collecting samples; d) Mrs. A. Z. Batten and Mr. G. R. Norman for technical assistance; and (e) Dr. T. J. Henderson of Rogar STB of London, Ontario, for supplying the vaccine.

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